

TITLE IMMUNOPRECIPITATION OF HPTK6

Project No. _____
Book No. 19211

Exhibit R, pg. 1 of 5

From Page No. _____

21st Jan

2 plates each of cells (CHO + FL-16 h1S) labelled w/ Cys + Met
for ~ 5 hours in serum ~~free~~ free DMEM (-Cys -Me)

- Pilled off media; took off cells in DMEM EDTA/
PBS for 10-15 min at room temp.; combined the 2 plates of
each; Washed cells 2x in PBS (5mls)

Packed cell volume of each sample ~ 50-100 μ
froze packed cell pellet -20°C

25th Jan

Thawed cells on ice and added
1ml of RIPA buffer + 1mM PMSF + Aprotin.

Allowed cells to lyse at 0°C for 30 min with
occasional vortexing.

Spin 5 min 11000 rpm in microfuge.

Sups removed to new tubes;

precleared lysate by adding 50% of Protein A-Sepharose

/INET slurry (1:1 mix) - 4°C on rotator 30 min.

To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Blw

26th Jan 94

From Page No. _____

Spin ; Sps to 2x fresh tubes (0.5 each)

added 0.5ml of RIPA + PMSF + Aprotin.

added 10 μ l of sera ; (Pre-spun sera)

Tube	Sample	Serum	Expt
1	CHO	8/11/93 B	Pre
2	CHO	11/18/93 B	Imm
3	FL-16 hiS	8/11/93	Pre
4	FL-16 hiS	11/18/93	Imm

; 1 hour 4°C labquake.

- Spin fast 5-10 min ; Sps to new tubes

. Added 50 μ l of Prot A-Sph Slurry.

~ 2 hrs at 4°C labquake.

Spin ; Sps removed + saved.

Pellets washed 4x w/ RIPA buffer

(2x 1ml, 2x 0.5ml) - each wash ~ 5-10 min.

~~Kerr~~

To Page No. _____

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

AP. PAN26th Jan 04

TITLE

IMMUNOPPT of HPTK6.

Project No. _____
Book No. 19211

Exhibit R, pg. 3 of 5

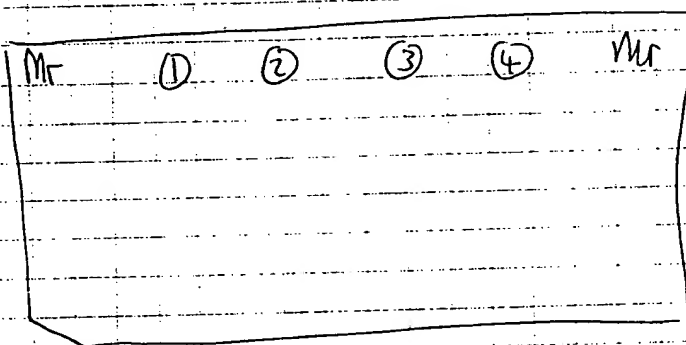
From Page No. _____

Added 150 λ of 1x Sample buffer (+ β -ME).

Heat 95°C 5-10 min w/ vortexing.

Spin out ProtA-Spn. beads.

loaded onto 8% PAGE.



- Ran slowly o/n.

Direct gel + Aut radi on BAS imaging Plate.

RESULTS & CONCLUSIONS.

There is a clear band visible in the FL-16 hiS cells that does not appear to be in the CHO cells alone. The band is approx. right mol wt; it is seen only in the immune serum and not the pre-immune.

To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

26 Jun 94

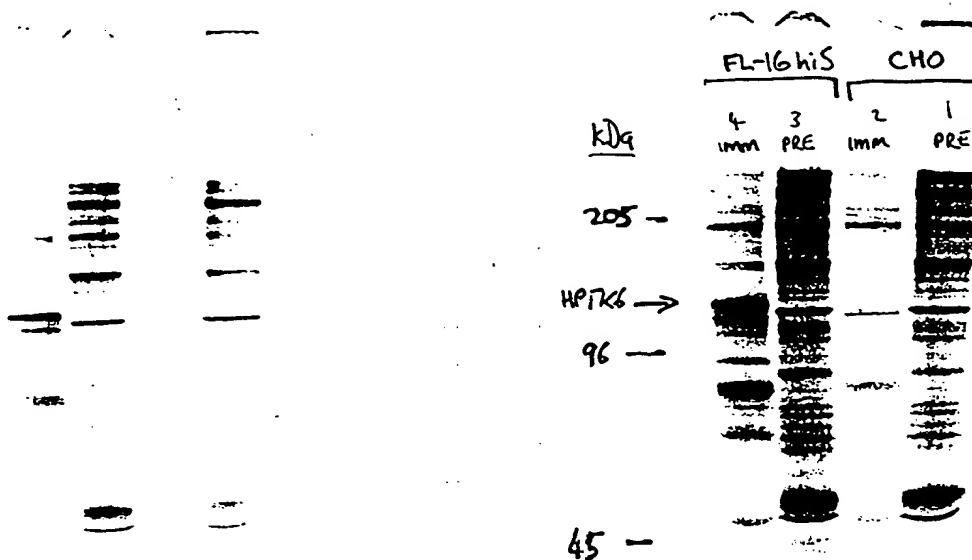
[Signature]

40

Project No. _____

Book No. 19211 TITLE _____

From Page No. _____



90 MIN EXP ON RAS

To Page No. _____

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

26th Jun 94

